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14. ABSTRACT

Magneto-rheological Fluid (MRF) is synthesized from suspensions of micron size iron particles in phosphate buffered saline (PBS). The iron particles are surface coated using atom transfer radical polymerization (ATRP) with various polymers, such as poly(N-isopropylacrylamide) (poly(NIPAAm)), and poly(acrylamide) (poly(AAm)). The surface grafted polymer has been characterized using differential scanning calorimetry (DSC), and properties of resulting fluid has been measured using a rheometer. A mathematical model is developed to explore the behavior of iron particles injected into tumor under magnetic field. Results showed that stress on the neighboring tissue is increased four times when the magnetic field is doubled from 0.2 Tesla to 0.4 Tesla. The effect of MRF on tumor growth are evaluated by using an orthotopic murine breast cancer model (4T1) by growth measurements and histological changes following injection of MRF or carrier fluid alone into the tumor and the effects of subsequent application of a magnetic field to the site. Therapy resulted in slowed tumor growth and significantly increased frequency of activated dendritic cells in the tumor but not in the spleen, suggesting a localized immune response. Also, MRF and magnetic field treatment inhibited the growth of orthotopic 4T1 tumors.

15. SUBJECT TERMS
Cancer therapy by localized immune response, Magneto-rehological Fluids

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INTRODUCTION

The main goal of this research is to assess the efficacy of augmenting immune responses to breast cancer through the use of magneto-rheological fluid (MRF), suspensions of micron size ferrous particles in a carrier medium. It is hypothesized that tumor may be damaged, even killed, by injecting a biocompatible MRF into the tumor and by applying an external magnetic field resulting in temporary semi-solid aggregate formation within the tumor. This may initiate of "danger" signals (i.e. pro-inflammatory cytokine production) resulting from the locally high stress on the neighboring tissue due to attraction forces between the ferrous particles caused by the magnetic field applied and tumor disruption. The specific aims of the study are to: 1) Design and characterize biocompatible MRF with coated particles; 2) Develop computational model to explore the behavior of iron particles injected into tumor under magnetic field and maximize their response while minimizing the particle size, concentration to improve biocompatibility and biodegradability 3) Evaluate the effect of MRFs on tumors injected with MRF and immune responses to the tumor using animal models.

BODY

Background

Traditionally, means to induce breast tumor regression has involved the use of surgery alone or with cytotoxic agents. However, chemotherapy and radiation therapy can kill off the immune effector cells as well as the tumor. Surgery results in removal of tumor burden such that the antigen pool is lost. Thus an alternate means therapy is needed if we wish to harness the potential of the immune system to help eliminate metastatic breast cancer. Injection of MRF into a primary tumor, followed by application of a magnetic field to the site may act augment immune responses to breast cancer through the induction of tumor death due to mechanical disruption of the tumor architecture. This will allow for antigen uptake, generation of "danger" signals allowing for augmentation of immune responses. Ultimately, this will allow for immune responses to disseminated disease. This combination of engineering, nanotechnology and immune attack represents a novel means to attack the cancer which could be applied to primary tumors such that disease eradication is possible.

Many types of controlled radical polymerization have been studied for surface polymerization of an inorganic substrate, such as atom transfer radical polymerization (ATRP). The application of ATRP for surface polymerization of iron particles has been investigated by Fuchs et. al. [1]. Surface modified iron particles were used in magnetorheological fluid (MRF) and magnetorheological elastomer (MRE) applications [2]. In the present work, MRFs were synthesized from suspensions of iron particles in carrier fluids, which contain phosphate buffered saline (PBS). The iron particles have been surface coated using atom transfer radical polymerization (ATRP) with various polymers, such as poly(N-isopropylacrylamide) (poly(NIPAAm)), and poly(acrylamides) (poly(AAm)). In addition, the simulant material for breast cancer has been synthesized using silicone gel.

Breast cancer tumor is a viscoelastic material with about 3kPa shear modulus [3]. Understanding the dynamics of particles under magnetic field is essential to predict their behavior after injection of MRF into a tumor and to choose the proper particle size and concentration. Controllable magnetic force attracts the particles toward the region of highest field gradient which are the poles of the magnet [4, 5]. Force-displacement relationship for a spherical particle in an elastic media was developed by Lin et. al. [6]. These forces, together with other forces such as particle-particle interaction have been used to model dynamic behavior of particles injected into a tumor under a magnetic field and the resulting stress on the neighboring tissue. A similar model was developed to study particle and flow dynamics in 2-dimensional micro channels recently by our group [7].

Hypothesis and Specific Aims

It is hypothesized that the injection of MRF intra-tumorally followed by magnetic treatment will result in necrotic tumor death by mechanical injury, release of tumor antigen and activation of localized inflammatory response. This hypothesis is tested by the fallowing specific aims:

1- MRF Synthesis: Design and develop MRF with desired rheology for the proposed study.

- Iron particle surface coating by atom transfer radical polymerization (ATRP) with various polymers.
- Characterization of surface coating and MRF using differential scanning calorimetry, and rheometer

2- Mathematical model: Develop a computational model to simulate the behavior of iron particles injected into tumor under magnetic field

- Motion of particles inside the elastic media.
- Stress applied by the particles on the elastic media (tumor)

3- Orthotopic 4T1 Mammary Cancer Model: Determine the effects of MRF and magnet treatment on:

- Immediate and late histological changes (i.e. necrosis, edema, cellular infiltration).
- Induction of apoptotic and stress responses in the primary tumor.
- Innate and adaptive immunological responses to the tumor

Results

MRF Synthesis Characterization

Iron particles were washed with distilled water and ethanol. ATRP was used to attach a polymer at the surface of the iron particles. The particles were dried in a vacuum oven at 50 °C and under nitrogen for 24 hours and cooled down. Dried iron particles were reacted at 85 °C with CTCS for 24 hours under nitrogen with toluene as a solvent. The mixture was then filtered and washed with methanol in order to remove excess CTCS. The residual (Fe-CTCS) was dried in a vacuum oven at ~40-50°C for 24 hours. Then, functionalized Fe-CTCS was reacted with CuBr, CuBr2, Spartein, and monomer in organic solvent at 85 °C (25 °C for NIPAAm) for 24 hours under nitrogen. Finally, the mixture was filtered, washed several times with ethanol and dried in a vacuum oven at ~40-50°C prior to use. Figure 1 shows the surface polymerization process.

Figure 2 shows the Differential Scanning Calorimetry (DSC) result of surface grafting of various polymers. These results indicate the presence of polymer on the surface of iron particles and the individual thermal transition temperature, signified by large change of heat supplied (heat flow endo up – Y-axis). Glass transition temperatures for Poly(NIPAAm) and Poly(AAm) are 235 °C and 218.7 °C, respectively. Corresponding values reported in the literature are 135 °C [8] and 196 °C [9]. Differences between literature and experiment are probably caused by the decreased mobility of the polymer due to the covalent bonding on the surface of the iron particles.

Rheology of breast cancer simulant materials is presented in Figure 3. Silicone gels with different concentrations of plasticizers were tested for their dynamic modulus and viscosity. The silicone gel with 4.35% plasticizer is found as the composition with matching shear modulus with the value reported in literature for breast cancer tumor [3].

Mathematical Model

Dynamic behavior of 2-micron iron particles inside an elastic media is modeled computationally. Initial location of the sixty spherical iron particles are randomly distributed inside the elastic medium (0 Tesla case). Their motion under the influence of magnetic, elastic and inter-particle forces and resulting stress on the elastic media is calculated. Shear modulus of the elastic medium is selected as the value reported for human breast cancer tumor [3]. Location of the particles after application of 0.4 Tesla magnetic field resulting from a permanent magnet at two simulation times are shown, 7.5 ms and 15 ms. Simulation results are demonstrated in Figure 4. Comparison of figures illustrates the dynamic motion of the particles. Resulting stress on neighboring tissue for 0.4 Tesla magnetic field is predicted to be about four times higher than the 0.2 Tesla case.

Animal Studies

4T1 breast cancer cell were implanted s.q. into the mammary fat pad of BALB/c mice. Cartoon representation of the experimental details is shown in Figure 5. Treatment was initiated when average tumor volume reached 340 mm3. Mice received 60% MRF (weight) in 100µl PBS or PBS alone. One group was then treated by placing a 0.5 Tesla magnet over the tumor for 10 min. per day for 5 consecutive days. Results indicated slower tumor growth after MRF implantation and magnetic field treatment (Fig. 6)

Histology of tumor at site of MRF injection before and after magnetic field application is shown in Figure 7. 2 x 10^5 4T1 cells were injected s.c into the mammary fat pad of female BALB/c mice. When tumors reached 300-350 mm^3, 100 µl of 60% MRF w/v in PBS was injected into the tumor. One group was treated for 10 min with a 0.5 Tesla magnet starting 24 hours after MRF injection for 5 consecutive days. All sections were stained with H &E. Original magnification is 400x. Images were captured with a Nikon E400 microscope equipped with a 100x numerical aperture objective lens. Image acquisition was performed with a Nikon Coolpix 5400 digital camera. Purple color represents nuclei and pink color represents cytoplasm. Tissue destruction appears as white areas and shrinkage of cells (MRF & Magnet). Iron appears as black particles

Frequency of activated dendritic cells in the tumor for the control group, for the groups injected with MRF, with and without magnet treatment are shown in Figure 8. 4T1 breast cancer cell were implanted s.c into the mammary fat pad of BALB/c mice. When tumors reached 300-350 mm^3, 100 µl of 60% MRF w/v in PBS was injected into the tumor. One group was treated for 10 min with a 0.5 Tesla magnet starting 24 hours after MRF injection for 4 consecutive days. Spleen and tumor cell suspensions were incubated with antibodies with R-phycoerythrin or PE (CD83), PE-cyanine or PC5 (MHC II), and PE-cyanine 7 or PC7 (CD11c+ SA). Listmode data files were collected on a five-color FC 500 MPL (Beckman Coulter). All data sets were analyzed using FlowJo software (TreeStar). Results indicate increased frequency of activated dendritic cells in the tumor after MRF and magnet treatment.

KEY RESEARCH ACCOMPLISHMENTS

- Biocompatible Magneto-rheological Fluids (MRF) are synthesized from iron particles coated with polymers and phosphate buffered saline (PBS) solution
- A computational model is developed to investigate the dynamic behavior of MRF injected into a tumor with the external magnetic field application
- Animal studies indicated that MRF and magnetic field treatment significantly increased the frequency of activated dendritic cells in the tumor but not in the spleen, suggesting a localized immune response. MRF and magnetic field treatment inhibited the growth of orthotopic 4T1 tumors.

REPORTABLE OUTCOMES

Myriam N. Bouchlaka, Alan Fuchs, Cahit A. Evrensel, Lisbeth A. Welniak and William J. Murphy, Mechanical disruption of the primary tumor using biocompatible magnetic beads in combination with immunotherapy allows for systemic anti-tumor responses in metastatic breast cancer, International Society for Biological Therapy of Cancer (iSBTc) 23rd Annual meeting, San Diego, October 31 - November 2, 2008

Evrensel C.A., Welniak L., Fuchs A., Murphy W.J., Gordaninejad F., Bouchlaka M., Sutrisno J., Patel J., Pyne T., Immune Response Augmentation in Metastasized Breast Cancer by Localized Therapy utilizing Biocompatible Magnetic Fluids, *Proceedings of the Era of Hope 2008*, Baltimore, June 2008.

CONCLUSION

MRFs for cancer therapy were synthesized from iron particles and PBS solution. Iron particles were surface modified using several polymers via ATRP technique. The thermal transitions of surface grafted polymer were higher than non-grafted polymer due to the decreased mobility of the polymer caused by covalent bonding.

A simulant for a cancer tumor was prepared using silicone gel with shear modulus close to the literature value for breast cancer tumor. It will be used to experimentally (at least qualitatively) study the behavior of iron particles inside the tumor under magnetic field.

A computational model was developed to investigate the dynamic behavior of particles injected into an elastic media with properties of a breast tumor. Model predicts that doubling the magnetic field (from 0.2 Tesla to 0.4 Tesla) almost quadruples the stress applied by the particles located close to surface on the neighboring tissue.

Animal studies indicated increased tumor destruction at the site of MRF implantation in tumors that were treated with daily application of a magnetic field. MRF and magnetic field treatment significantly increased the frequency of activated dendritic cells in the tumor but not in the spleen. These data suggest the therapy induced a localized immune response. MRF and magnetic field treatment inhibited the growth of orthotopic 4T1 tumors. We predict that this treatment, in combination with other immunotherapies, will greatly enhance the immune response to both localized and metastatic disease

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SUPPORTING DATA

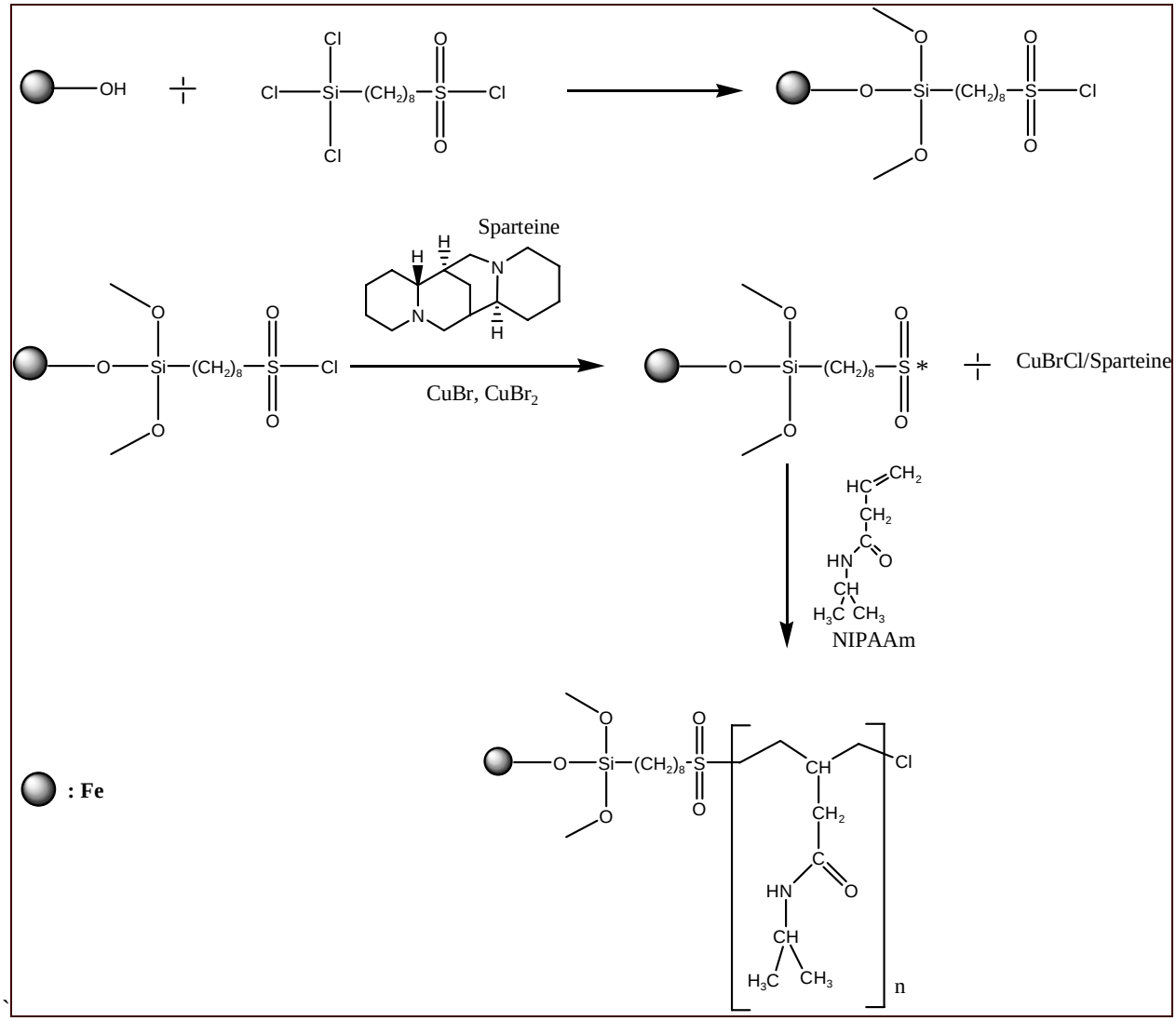


Figure 1 Surface polymerization poly(NIPAAm) on the iron particles using ATRP.

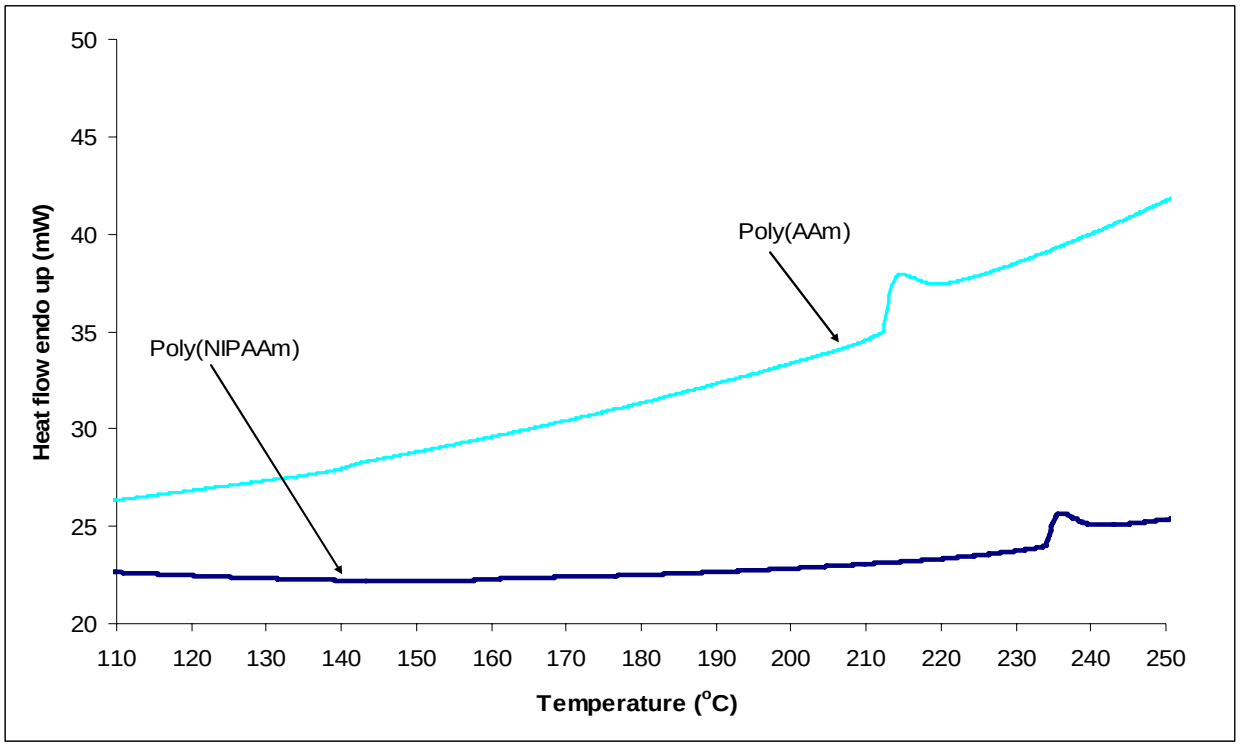


Figure 2 DSC result of surface grafting of various polymers**.**

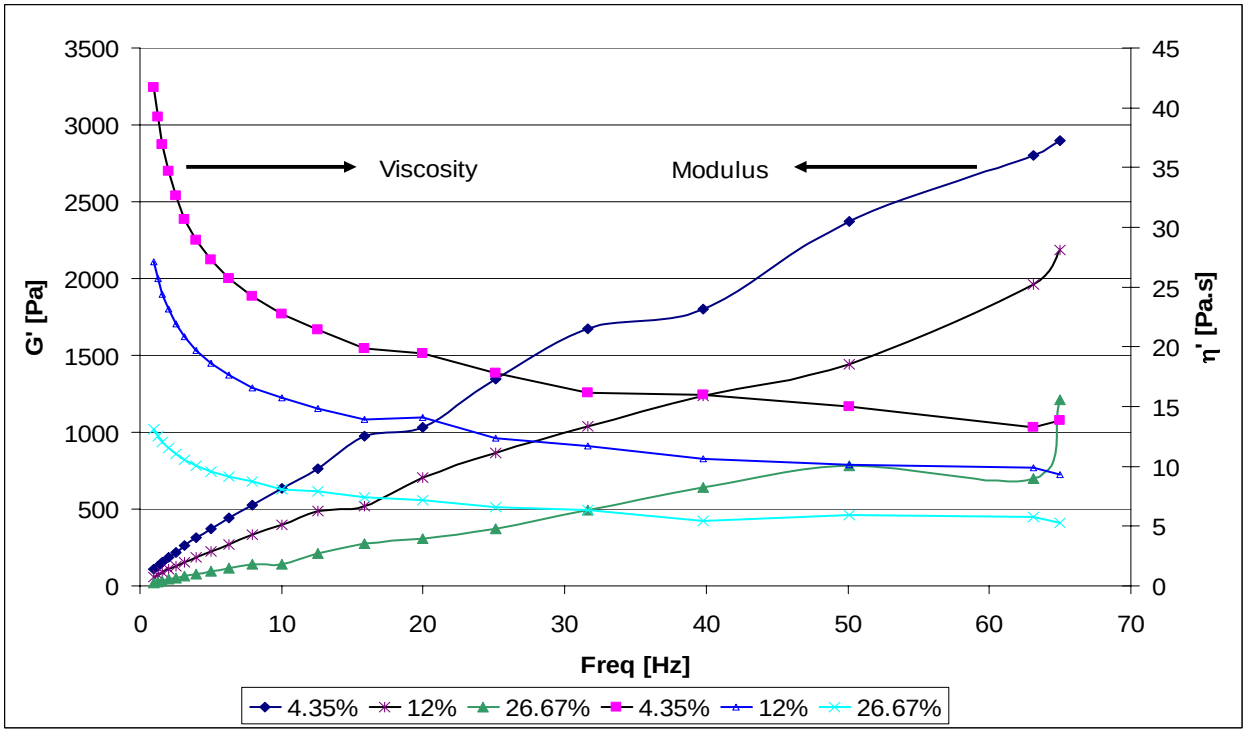
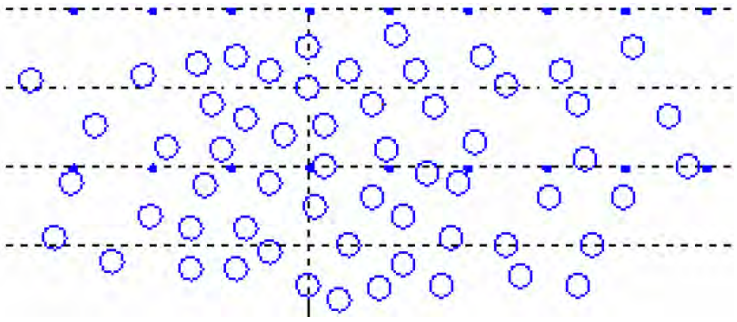
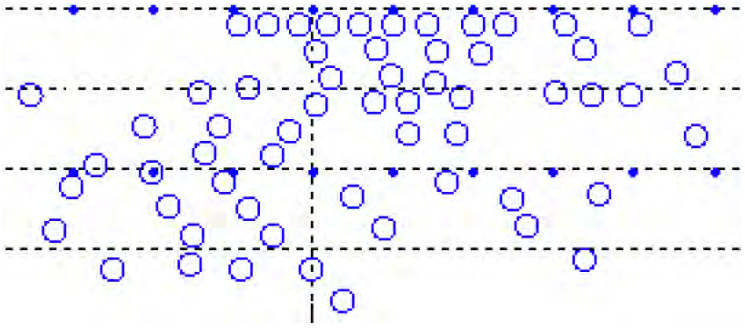


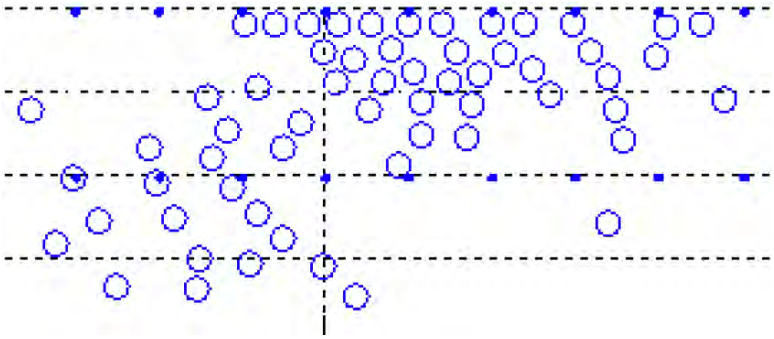
Figure 3 Rheology of breast cancer simulant materials.



a) T=0 sec, 0 Tesla (no magnetic field)



b) t = 7.5 ms, 0.4 Tesla



c) t = 15 ms, 0.4 Tesla

Figure 4. Dynamic behavior iron particles inside an elastic medium under magnetic field

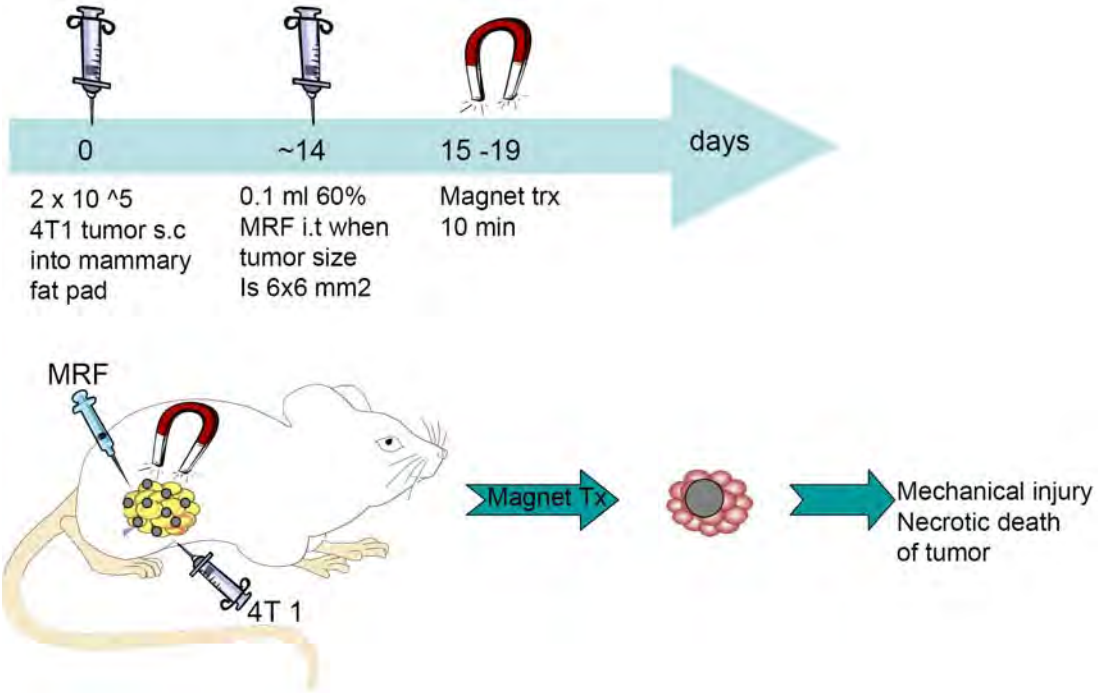


Figure 5. Method, animal study

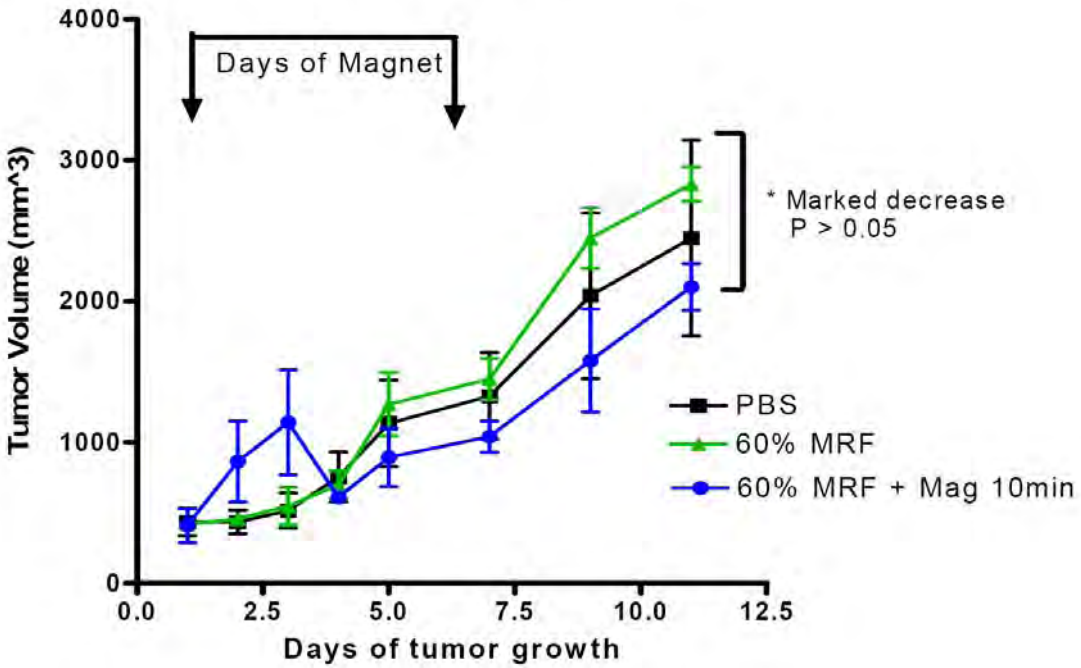


Figure 6 . Slower Tumor growth after MRF implantation and magnetic field treatment.
*2 way ANOVA p>0.05

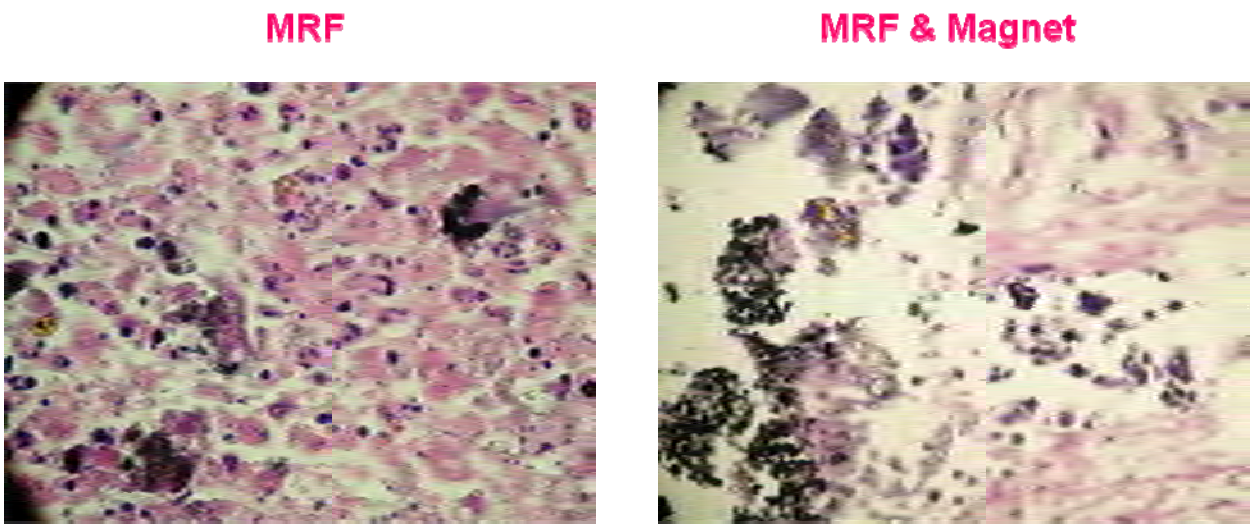


Figure 7: Histological examination of tumor at site of MRF injection.

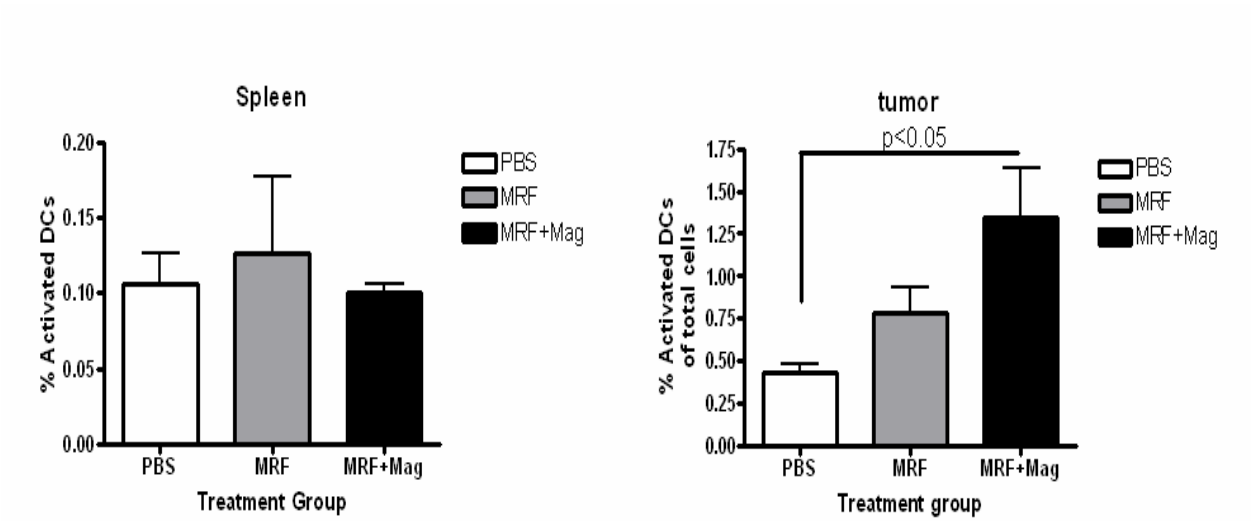


Figure 8: Increased frequency of activated dendritic cells in the tumor after MRF and magnet treatment.